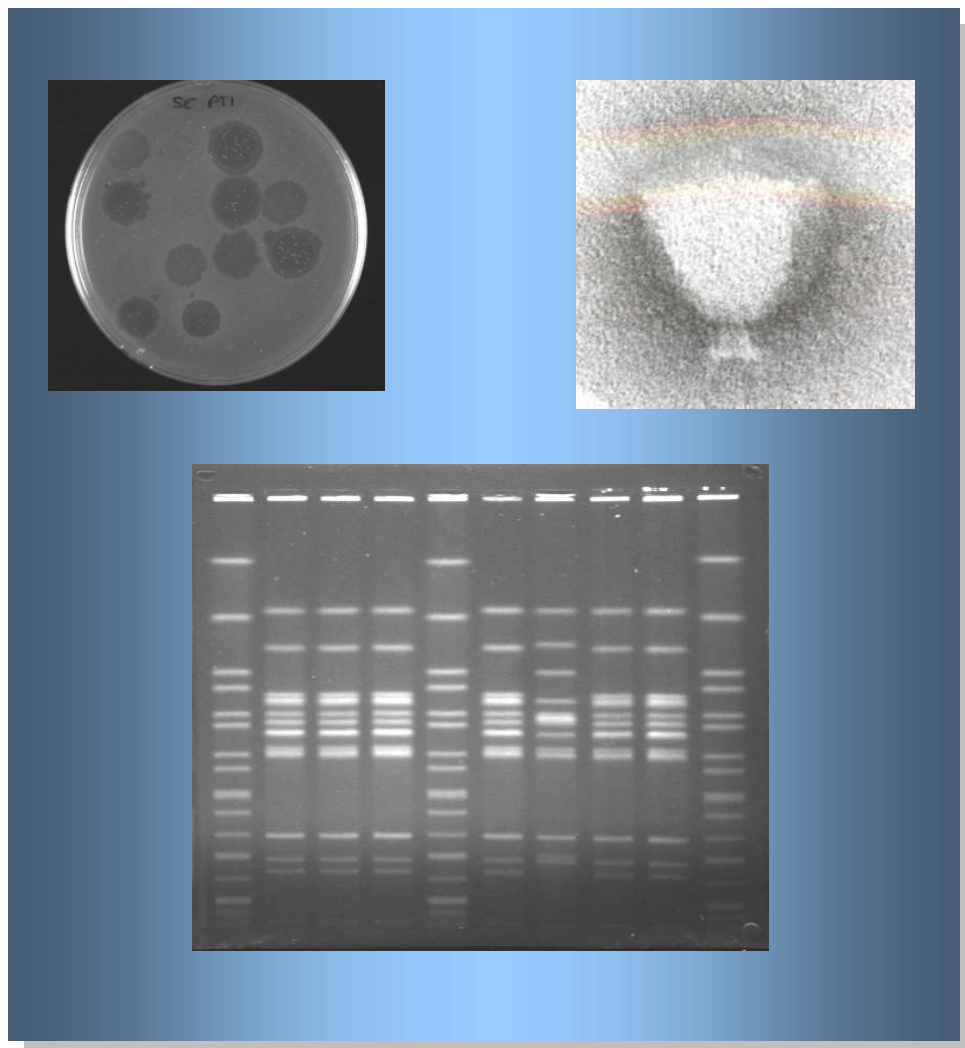


**NATIONAL *SALMONELLA* REFERENCE LABORATORY OF IRELAND
(HUMAN HEALTH)**



ANNUAL REPORT FOR 2009



NATIONAL *SALMONELLA* REFERENCE LABORATORY

Introduction

The National *Salmonella* Reference Laboratory was established in 2000 with support from the Department of Health and Children to provide reference services related to human health. It is a public service laboratory with 3 full time scientific staff. It operates from the Department of Bacteriology at the Clinical Science Institute, NUI Galway. The NSRL website is at http://www.nuigalway.ie/salmonella_lab/. The reference laboratory uses a number of phenotypic methods (serotyping, antibiotic-resistance testing and phage typing) and molecular methods (pulse-field gel electrophoresis (PFGE) and multi-locus variable number tandem repeat analysis (MLVA) to precisely characterise *Salmonella* isolates. This process can be considered as genetic fingerprinting of *Salmonella*. The goal of this genetic fingerprinting is to assist relevant agencies in protecting public health by identifying and interrupting chains of transmission of *Salmonella* infection. In addition the NSRL provides typing services for *Shigella species* and *Listeria monocytogenes*.

A particular phenomenon that has become very prominent in Ireland in 2009 is the increase in the number of isolates that represent a monophasic variant of *S. Typhimurium*. Cells of *S. Typhimurium*, as with most other serovars of *Salmonella*, have genes for 2 different types of flagella (called phase 1 flagella and phase 2 flagella). In *S. Typhimurium* the phase 1 flagellar antigen is “i” and the phase 2 flagellar antigen is “2”. There is a process of genetic switching (variation between 2 “phases”) in *Salmonella* so that a culture of *S. Typhimurium* comprises a mixture of individual cells some of which are expressing “i” and some of which are expressing “2”. There are variants of *S. Typhimurium* that only have the capacity to express one type of flagellar antigen (monophasic variants) so that all cells in the culture express a single common flagellar antigen. Such monophasic variants have long been recognised but have been relatively uncommon. This situation has changed quite dramatically in recent years with increasing numbers of monophasic *S. Typhimurium* in Ireland and throughout Europe. The biological basis for this change is the subject of significant research. The growth of this phenomenon in Ireland is referred to below.

The Laboratory is committed to providing a high quality and timely service and has been inspected for accreditation to the ISO15189 standard from the Irish National Accreditation Board (INAB) and is awaiting a final decision. The continued success of the laboratory is entirely dependent on the support of the staff in the laboratories that submit isolates for typing. My colleagues and I appreciate that the preparation, packing and dispatch of isolates is a significant burden and would like to thank you for your support over the years.

I would also like to acknowledge the support of all those agencies with whom we work closely to ensure that the service we provide works as information for action. In particular I would like to thank the Food Safety Authority of Ireland, the Health Protection Surveillance Centre and colleagues in Public Health Departments and Environmental Health Departments throughout the country and to acknowledge the work of colleagues in the National Reference Laboratory *Salmonella* (Food, Feed and Animal Health)¹.

If you have any comments or questions arising from the report please feel free to contact me at the email address given below.

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1. The Annual Report of National Reference Laboratory *Salmonella* (Food, Feed and Animal Health) is available at

<http://www.agriculture.gov.ie/media/migration/animalhealthwelfare/labservice/nrl/NRLAnnualReport2009.pdf>

Ireland

In 2009, 810 isolates were submitted to the National *Salmonella* Reference Laboratory. When non-*Salmonella*, QC contaminants and duplicate isolates were removed a total of 732 *Salmonella* isolates were typed. This represents a 42 % decrease in the number of isolates received in 2008.

There were 364 human clinical isolates, including 323 faecal isolates, 24 from blood (including 9 *S.Typhi*, 7 *S.Paratyphi A* and 2 *S.Mbandaka*), 4 other invasive isolates, 11 urine isolates and 2 from unspecified sites. *S.Typhimurium* (n = 87) and its monophasic variant 4,5,12:i:- (n =31) and *S.Enteritidis* (n = 87) predominated (Table 2). There was marked seasonal variation with the highest number of isolates occurring in months May to October. This coincides with the warmer months of the year and with the peak season for foreign travel (Fig.1) and may be related in part to one or both of these factors.

In some cases more than one isolate was received from a patient. For example we may have received an invasive isolate (e.g. from a blood culture) and an isolate from faeces from the same patient. Where invasive and faecal isolates come from the same patient, only the invasive isolate is recorded to avoid duplication. The average turnaround time for testing was 6 days (range 1-28 days). There was an 18% decrease in the number of *Salmonella* isolates from humans received in the NSRL compared with 2008 (Table 1).

Table 1: Number of *Salmonella* isolates received in NSRL

Year	Human	Non-human
2009	364	368
2008	447	815
2007	457	653
2006	430	308
2005	357	494
2004	420	650
2003	486	634
2002	394	540

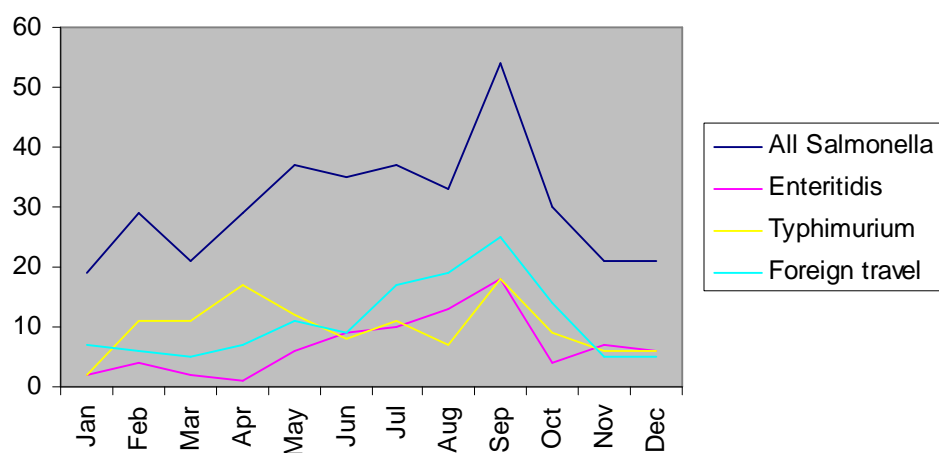
2001	508	574
2000	636	214

Table 2: Top fifteen serotypes of Human Isolates (inc typhoidal)

Serotype	Frequency	%
Typhimurium	87	26
¹ 4,5,12:i:-	31	9
Enteritidis	87	26
Typhi	9	3
Paratyphi A	9	3
Kentucky	7	2
Agona	6	2
Java	6	2
Dublin	6	2
4,5,12:b:-	6	2
Bredeney	5	1.5
Virchow	5	1.5
Saintpaul	5	1.5
Mbandaka	5	1.5
Newport	4	1
Others	86	17
Total	364	100

¹ This antigenic formula is that of *S. Typhimurium* except that the phase 2 antigen is not expressed. These isolates are generally referred to as monophasic *S. Typhimurium*.

Figure 1 Seasonal Variation in *Salmonella* isolates ¹



1. The line “Foreign Travel” describes the number of cases of salmonellosis for which an association with recent foreign travel was reported to NSRL. Reporting of recent foreign travel is likely to be incomplete.

It is important to note that there is always an interval gap between the time of onset of symptoms and date of isolate receipt in the NSRL. This includes time taken for patient to access doctor, taking and transporting the sample to the primary laboratory, isolation of *Salmonella*, and referral to NSRL.

Salmonellosis non-typhoidal

*S.*Typhimurium and its monophasic variant.

*S.*Typhimurium 4,5,12; i: 2 and its monophasic variant (4,5,12;i:-) together accounted for 35% of all cases of *Salmonella*. Phage types DT104 accounted for 20%, DT104b 12% and DT193 accounted for 23% of the total number of *S.*Typhimurium/4,5,12;i:- isolates. The predominance of phage type DT104 and related phage types are similar to recent years. The increase in numbers of the monophasic variant is striking and is discussed in more detail below.

*S.*Enteritidis

*S.*Enteritidis accounted for 26% of all cases of *Salmonella*. The predominant phage types were PT14b (23%), PT8 (15%), PT21 (13%), PT1 (10%) and PT4 (8%). The increase in PT14b was primarily caused by an outbreak (n = 10) of nalidixic acid resistant PT14b.

Salmonellosis Typhi and Paratyphi

Nine isolates of *S.Typhi*, 9 isolates of *S.Paratyphi A* and 2 isolates of *S.Paratyphi B* were received. A history of recent travel was recorded for 7 of the *S.Typhi* isolates; 6 to the Indian subcontinent and 1 to the Phillipines. Seven of the *S.Paratyphi A* isolates were associated with reported travel to the Indian subcontinent, while one isolate had foreign travel with country not stated. A history of travel to South America (country not stated) was recorded for the *S.Paratyphi B* isolates.

Antimicrobial resistance

Fifty percent of isolates were susceptible to all antimicrobial agents tested. Twenty-eight percent of isolates were multi-drug resistant (four or more antibiotics). Of the 28% of multi-drug resistant isolates, 40% had the profile of resistance to ampicillin, chloramphenicol, streptomycin, sulphonamide and tetracycline (ACSSuT) and were mainly *S.Typhimurium* DT104 or closely related phage types. This group of *S. Typhimurium* DT104 has accounted for a high proportion of multidrug-resistant *Salmonella* isolates for some years now.

Three extended spectrum beta-lactamase (ESBL) producing isolates were detected. All were from patients with a history of foreign travel including *S.Concord* from a child from Ethiopia and *S.Typhimurium* isolates from patients with travel history to Italy and Morocco. The association of ESBL producing *S. Concord* with children adopted from Ethiopia has been noted in a number of countries and a report of an international collaborative study on this phenomenon in which NSRL participated was published in 2009.

Six isolates of *Salmonella* resistant to ciprofloxacin were detected. Five of these were from an *S.Kentucky* outbreak associated with a caterer while the other was a *S.Indiana* isolate with no report of foreign travel. In addition fifty isolates resistant to nalidixic acid and with reduced susceptibility to ciprofloxacin were detected; a decrease from 68 in 2008.

Travel related infection

A history of recent foreign travel was recorded in 35% (129) of human cases of infection (Table 3). Spain was the most commonly recorded travel destination (recorded in 24 cases) with *S. Enteritidis* (n = 17) accounting for the majority of these cases. More than half

(55%) of *S. Enteritidis* isolates were associated with foreign travel compared to 15% for *S. Typhimurium* and its monophasic variant combined. Although NSRL does not have access to data on the number of Irish people who travel to each country it is likely that the number of cases associated with each country is at least in part accounted for by the popularity of the country as a holiday destination.

Table 3: Foreign travel history for *Salmonella* isolates

Continent	Country	Number
Europe (n = 52)		
	Spain	24
	Portugal	6
	Turkey	3
	Croatia	3
	UK	3
	Bulgaria	3
	Malta	2
	Italy	1
	Poland	2
	Cyprus	1
	Greece	1
	Hungary	1
	Romania	1
	The Netherlands	1
Africa (n = 30)		
	Nigeria	11
	Morocco	2
	Sudan	2
	Egypt	1
	Tanzania	2
	S.Africa	2
	Ghana	1

Kenya	1
Ethiopia	1
Madagascar	1
Tunisia	1
Zambia/Botswana	1
N.Africa *	2
Africa *	2

Australasia (n = 33)

India	10
Bangladesh	3
India/Bangladesh	1
Pakistan	4
Thailand	3
Mauritius	2
Vietnam	1
Vietnam/Thailand	1
Malaysia	1
Australia	1
Philippines	1
China	1
Bahrain	1
Bali	1
Bali/Singapore	1
Maldives	1

Americas (n = 10)

USA	5
Mexico	1
Trinidad & Tobago	1
Brazil	1
South America	2

4 Unknown

* Country not stated

Clusters

Twenty-eight clusters of cases involving 106 isolates were identified in 2009. *S.Typhimurium* was involved in 16 clusters while *S.Enteritidis* was implicated in 4 clusters.

Nine of the clusters were family outbreaks, i.e. all patients affected were from one family, while 7 outbreaks were associated with foreign travel (all patients had travelled to same country).

A point source outbreak involving both a multi-resistant (ASSuTNaCpGm) *S.Kentucky* (n = 5) strain and an *S.Agona* (SSuT) strain (n = 4) was traced to a catering establishment. The *S.Agona* strain had a different antibiogram and pulsed field profile (PFP) to that from the 2008 *S. Agona* outbreak which was linked to a food production plant in Ireland.

There was an increase in the number of *Salmonella* 4,5,12:i:- (monophasic *S.Typhimurium*) DT193 human infections in 2009. These had a distinct PFP, i.e. STYMXB0131 and had resistance to ampicillin, streptomycin, sulphonamide and tetracycline (ASSuT) or some variant of this (such as SSuT or T). MLVA proved useful in separating these isolates into 3 clusters; E1 (n = 9) = 3-11-10-NA-211, from the East of the country; E2 (n = 10) = 3-13-9-NA-211, which was geographically dispersed and E3 (n = 2), 3-13-9-NA-211, a family outbreak associated with consumption of well water. Typing of isolates from animal sources showed similarities with swine isolates while an isolate from well water was indistinguishable from that from the E3 outbreak. The outbreak was investigated by relevant agencies with microbiological support from NSRL. A link with dog ownership and the use of pig ear treats was suspected but could not be confirmed.

Five isolates of a rare *S.Typhimurium* phage type, DT8, with MLVA pattern 2-10-NA-12-212, were typed in the NSRL from August to December 2009. This phage type is associated with ducks and several of the cases were linked epidemiologically with ducks or consumption of duck eggs. A similar outbreak occurred at the same time in Scotland which

was also linked to ducks. This episode relates to further problems with this strain currently being managed in 2010.

The NSRL liaises with the European Centre for Disease Control (ECDC) in the investigation of outbreaks that may have an international dimension. The UK declared an outbreak of *S.Typhimurium* DT191a, resistant to tetracycline and with a distinct MLVA profile involving more than 100 isolates since August 2008. A case control study showed a strong association with reptile contact, especially snakes. The NSRL documented 4 of these strains in 2008 and 3 in 2009, 2 of which had contact with snakes.

From November 2009 the NSRL typed 11 isolates of *S.Enteritidis* PT14b with resistance to nalidixic acid. There was a similar rise in the UK which was linked to the consumption of Spanish eggs. Irish isolates have been provided to colleagues in the UK to assist in further research on this strain.

Evidence of Links between *S. Typhimurium* in Humans, Food and Animals.

Multi locus variable number tandem repeat analysis (MLVA) is a relatively new technology that allows for very fine discrimination between isolates that appear very closely related by other methods including PFGE. This precise discrimination has proved a useful technique in showing persuasive evidence of links between human *S.Typhimurium* clusters and individual cases and recent food and animals sources. Table 4 illustrates evidence of links between human infection and food /food animal types. In some cases the link is complete from animal, to animal related food product and to human cases.

The importance of using standardised and readily comparable methods for “fingerprinting” human, food and animal isolates of *Salmonella* not just within Ireland but across Europe and the world is illustrated by this body of work over the past year. Overall there is some further support for a link between swine and *S.Typhimurium* infection in humans suggested previously in the 2008 report from NSRL.

Table 4 MLVA profiles of human and food/animal isolates

Isolate no.	Source	Phage type	Resistance	MLVA profile
S09-0122	Equine	DT104	ACSSuT	3-13-15-13-311
S09-0080	Human	DT104	ACSSuT	3-13-15-13-311
S09-0123	Equine	DT104	ACSSuT	3-13-15-14-311
S09-0124	Equine	DT104	ACSSuT	3-13-15-14-311
S09-0125	Equine	DT104	ACSSuT	3-13-15-14-311
S09-0231	Human	DT120 ^{low}	ACSSuT	3-13-15-14-311
S09-0258	Human	DT104	ACSSuT	3-13-15-14-311
S09-0273	Human	DT104	ACSSuT	3-13-15-14-311
S09-0339	Human	DT104	ACSSuT	3-13-15-14-311
S09-0560	Human	DT104	ACSSuT	3-13-15-14-311
S09-0613	Human	DT104	ACSSuT	3-13-15-14-311
S09-0419	Swine	DT193	ASSuT	3-13-9-NA-211
S09-0633	Well water	DT193	ASSuT	3-13-9-NA-211
S09-0203	Human	DT193	ASSuT	3-13-9-NA-211
S09-0206	Human	DT193	ASSuT	3-13-9-NA-211
S09-0263	Human	DT193	ASSuT	3-13-9-NA-211
S09-0291	Human	DT193	ASSuT	3-13-9-NA-211
S09-0322	Human	DT193	ASSuT	3-13-9-NA-211
S09-0325	Human	DT193	T	3-13-9-NA-211
S09-0531	Human	DT193	ASSuT	3-13-9-NA-211
S09-0593	Human	DT193	ASSuT	3-13-9-NA-211
S09-0608	Human	DT193	ASSuT	3-13-9-NA-211
S09-0644	Human	DT193	ASSuT	3-13-9-NA-211
S09-0639	Human	DT193	ASSuT	3-13-9-NA-211
S09-0718	Human	DT193	ASSuT	3-13-9-NA-211

S09-0106	Swine	U311	ASSuT	3-14-10-NA-211
S09-0119	Human	U311	ASSuT	3-14-10-NA-211
S09-0144	Human	U311	ASSuT	3-14-10-NA-211
S09-0176	Swine	U311	ASSuT	3-14-10-NA-211
S09-0177	Swine	U311	ASSuT	3-14-10-NA-211
S09-0182	Swine	U311	ASSuT	3-14-10-NA-211
S09-0323	Swine	U311	ASSuT	3-14-10-NA-211
S09-0324	Swine	U311	ASSuT	3-14-10-NA-211
S09-0508	Swine	O rough:i:-	ASSuT	3-14-10-NA-211
S09-0509	Swine	O rough:i:-	ASSuT	3-14-10-NA-211
S09-0791	Bovine	U311	ASSuT	3-14-10-NA-211
S09-0782	Swine	DT104	ASu	3-14-6-12-311
S09-0783	Swine	DT104	ASu	3-14-6-12-311
S09-0810	Human	DT104	ASu	3-14-6-12-311
S09-0451	Human	DT104b	SSu	3-14-8-12-311
S09-0480	Human	DT104b	SSu	3-14-8-12-311
S09-0605	Swine	DT104b	SSu	3-14-6-12-311
S09-0097	Human	DT104	ACSSuT	3-16-7-7-311
S09-0373	Swine	DT104	ACSSuT	3-16-7-7-311
S09-0374	Swine	DT104	ACSSuT	3-16-7-7-311

* DT104, DT104b and DT120 are closely related phage types.

* Rough:i:- Isolate has mutation resulting in poorly expressed O antigen genes. Not possible to phage type but H antigens indicative of monophasic *S.Typhimurium* (4,5,12:i:-)

Animal Contact

A history of animal contact was recorded for 71 patients with salmonellosis including contact with terrapins, lizards, snakes, fish, pet birds, horses, dogs and farm animals (Table 5). Dogs were the most common contact animal (n = 32) while contact with cats was less common (n = 11).

In some cases typing of isolates from patients and individual animals they had contact with, e.g. horse (*S.Typhimurium* DT104), canary (*S.Typhimurium* DT135) and terrapins (*S.Kottbus* and 4,5,12:b:-), showed them to be indistinguishable. In addition to those cases where NSRL was aware of patient reported animal contact the molecular typing results in Table 4 above suggest that contact with horses may merit further consideration in assessing possible sources of human salmonellosis.

Other strong links included a 6 month old child with *S. II* 6,7:m,t and contact with a snake; 2 children with 4,5,12:i:- DT191a and a pet snake; 2 sisters with *S.Jangwani* and tropical fish; a 1 month old baby with *S.Monschaui* and a 4 month old baby with *S.Poona* and contact with lizards. Although public information on the risk (particularly to children) of contact with reptiles has been circulated it appears that this may not be reaching relevant sections of the population or may not have resulted in modification of risk behaviour. It may be appropriate to consider if further steps to limit exposure of children to risk of salmonellosis from contact with reptiles and other exotic pets is appropriate. In total 28 isolates of *Salmonella* (approx. 7.5% of all human cases) were associated with contact with exotic animals, predominately reptiles.

Among people that had a recorded history of living on or visiting farms (n = 13), *S.Typhimurium* predominated (n =11), especially the DT104 phage group. Some of these patients had contact with sick cattle.

Table 5: Animal contact history

NSRL no.	Source	Sub ¹ .	Strain	Animal Contact
S09-0080	Human	I	Typhimurium DT104	Horse
S09-0123-5	Horse	I	Typhimurium DT104	-
S09-0205	Human	I	Typhimurium DT135	Pet shop
S09-0445	Human	I	Typhimurium DT135	Pet Canary
S09-0467	Canary	I	Typhimurium DT135	-
S09-0353	Human	I	Kottbus	Tortoise, fishtank, Budgie
S09-0491	Tortoise	I	Kottbus	-
S09-0407	Human	I	4,5,12:b:-	Terrapin
S09-0468	Terrapin	I	4,5,12:b:-	-
S09-0470	Terrapin	I	4,5,12:b:-	-
S09-0002	Human	II	6,7:m,t	Snake
S09-0121	Human	I	Enteritidis PT4	Snake
S09-0295	Human	I	Enteritidis PT8	Snake, bearded dragon, turtle
S09-0359	Human	I	4,5,12:i:- DT191a	NS
S09-0626	Human	I	4,5,12:i:- DT191a	Snake, dog, cat, chicken
S09-0692	Human	I	4,5,12:i:- DT191a	Snake, dog, cat, chicken
S09-0732	Human	I	6,7:e,h:-	Snake
S09-0580	Human	I	Enteritidis PT8	Snake
S09-0055	Human	I	Hadar	Turtle

S09-0230	Human	I	O rough:y:5	Turtle, Bearded dragon, goldfish, dog
S09-0570	Human	I	Enteritidis PT1	Tortoise
S09-0597	Human	I	Enteritidis PT1	Tortoise
S09-0679	Human	I	Typhimurium DT24	Tortoise, lion, cheetah
S09-0130	Human	I	Enteritidis PT14b	Reptiles
S09-0223	Human	I	Monschau	Lizard
S09-0462	Human	I	Poona	Lizard
S09-0665	Human	I	Braenderup	Lizard
S09-0656	Human	I	Jangwani	Tropical fish
S09-0668	Human	I	Jangwani	Tropical fish
S09-0015	Human	I	Typhimurium Untypable	Farm
S09-0219	Human	I	Typhimurium DT104	Farm
S09-0276	Human	I	Typhimurium DT104b	Farm, cats
S09-0296	Human	I	Typhimurium DT104b	Farm, cats
S09-0328	Human	I	Typhimurium DT104b	Farm
S09-0339	Human	I	Typhimurium DT104	Farm, cattle sick, dogs
S09-0409	Human	I	Durham	Farm
S09-0472	Human	I	Enteritidis PT21	Pet Farm
S09-0531	Human	I	4,5,12:i:- DT193	Pet Farm
S09-0575	Human	I	Typhimurium DT104b	Farm, dogs
S09-0576	Human	I	Typhimurium DT8	Farm, poultry, ducks
S09-0760	Human	I	Typhimurium DT104	Farm, poultry, ducks
S09-0778	Human	I	Typhimurium DT104	Farm, sick calves
S09-0292	Human	I	Dublin	Father works in piggery
S09-0001	Human	IV	44:z4,z23	NS
S09-0035	Human	IV	48:g,z51	Farm animals
S09-0068	Human	IIIa	41:z4,z23	NS
S09-0391	Human	IIIb	48:z52:z	NS
S09-0591	Human	IIIb	61:k:z35	NS

1. Sub = Subspecies. There are over 2500 *Salmonella* serotypes of which approximately 60% belong to *subspecies* I. These account for about 99% of human infections. *Subspecies* I is present in both warm and cold blooded animals while the other *Salmonella subspecies* are generally associated with cold-blooded animals.

Non-Human isolates

In 2009, 368 isolates of *Salmonella* of non-human origin were submitted to the NSRL. This represents a decrease of 55% in the number of non-human isolates received in 2008. The majority of isolates were from swine (n = 155) and poultry (n = 110) while there were 28 isolates from bovine sources. *S.Typhimurium/4,5,12;i:-* (n = 146) and *S.Kentucky* (n = 88) were the most prevalent serovars (Table 6).

Table 6 Top fifteen serotypes among non-human isolates

Serotype	Frequency	%*
Typhimurium	123	33
4,5,12;i:-	23	6
Kentucky	88	24
Tennessee	20	5.5
Mbandaka	14	4
Agona	12	3
Senftenberg	10	2.5
Derby	9	2.5
Infantis	8	2
Dublin	5	1.5
Indiana	3	1
Virchow	3	1
Goldcoast	3	1
Others	47	13
Total	368	100

* Approximate figures

***Salmonella* serotypes and correlation with Human Infection**

*S.*Typhimurium

*S.*Typhimurium and its monophasic variant 4,5,12:i:- accounted for 39% of all non-human isolates and were isolated from a variety of sources predominantly swine (n = 124) but also including bovine (n = 10) and poultry (n = 1) sources. Phage types DT104b (n = 31), DT193 (n = 23), DT104 (n = 17), DT120/DT120 low (n = 12), U311 (n = 20), Untypable (n = 7) and U302 (n = 4), were the most common phage types from swine. Phage type DT104 (n = 6) was the most common phage type among bovine isolates. As illustrated in Table 4 (above) there is evidence from molecular typing of a significant link between isolates from pigs and pig products and human infection.

Salmonella Enteritidis.

A *S.*Enteritidis PT4 isolate was received from poultry (Brazilian chicken). It is of interest that *S.* Enteritidis is rarely detected from poultry or poultry products in Ireland but that *S.* Enteritidis is the first or second most common serovar isolated from human infections. In this context it is worth noting that a history of travel outside of Ireland is reported in relation to 55% of cases *S.* Enteritidis compared with only 12% of *S.* Typhimurium/4,5,12:i:-.

Sources of Isolates

Swine

A total of 11 serovars accounted for the 155 isolates received from swine with *S.*Typhimurium/ 4,5,12:i:- (n = 124) accounting for the majority of isolates.

Poultry

A total of 110 isolates comprising 12 serovars were received from poultry sources. *S.* Kentucky was the most common serovar (n = 84) and most isolates were susceptible to all antimicrobial agents tested. However 2 isolates were AmpC producers (CMY-2) with resistance profile ACazCtx and 3 were ESBL (SHV12) producers with resistance profile ACSuTCaz. No *S.*Kentucky isolates with this resistance profile were received in the NSRL.

Other common serovars from poultry sources include *S.Senftenberg* (n = 4; 1 human case), *S.Virchow* (n = 3; 5 human cases) and *S.Indiana* (n = 3; 1 human case).

Bovine

A total of 28 isolates were received from bovine sources. *S.Typhimurium* accounted for the majority of isolates (n = 10) while there were also 3 *S.Dublin* isolates (6 human cases).

Antimicrobial Resistance

Forty-six percent of isolates were susceptible to all antimicrobial agents tested. Thirty-nine percent were multi-drug resistant (four or more antibiotics). Thirteen percent of isolates had the profile of resistance to ampicillin, chloramphenicol, streptomycin, sulphonamide and tetracycline (ACSSuT) and most of these typed as *S.Typhimurium* DT104 or closely related phage types.

Resistance to third generation cephalosporins was identified in 5 *S. Kentucky* isolates from poultry received in the early part of 2009. These included 2 plasmid-mediated AmpC producers, CMY-2 (S09-0135 and S09-0171) and 3 ESBL-producers, SHV-12 (S09-0031, S09-0060 and S09-0061). These patterns were also seen in poultry in late 2008 and are the first reports of third generation cephalosporin resistance in *Salmonella* from food animals in Ireland and is a cause of concern. A detailed study of these isolates was published in 2009 by Boyle and colleagues. (**Boyle, F., Morris, D., O Connor, J., DeLappe, N., Ward, J., and M.Cormican (2010). First Report of Extended-Spectrum-B-Lactamase-Producing *Salmonella enterica* Serovar Kentucky Isolated from Poultry in Ireland. AAC. 54:551-553.**)

None of the isolates were resistant to ciprofloxacin; however 22 isolates exhibited resistance to nalidixic acid and reduced susceptibility to ciprofloxacin.

Laboratory Contamination

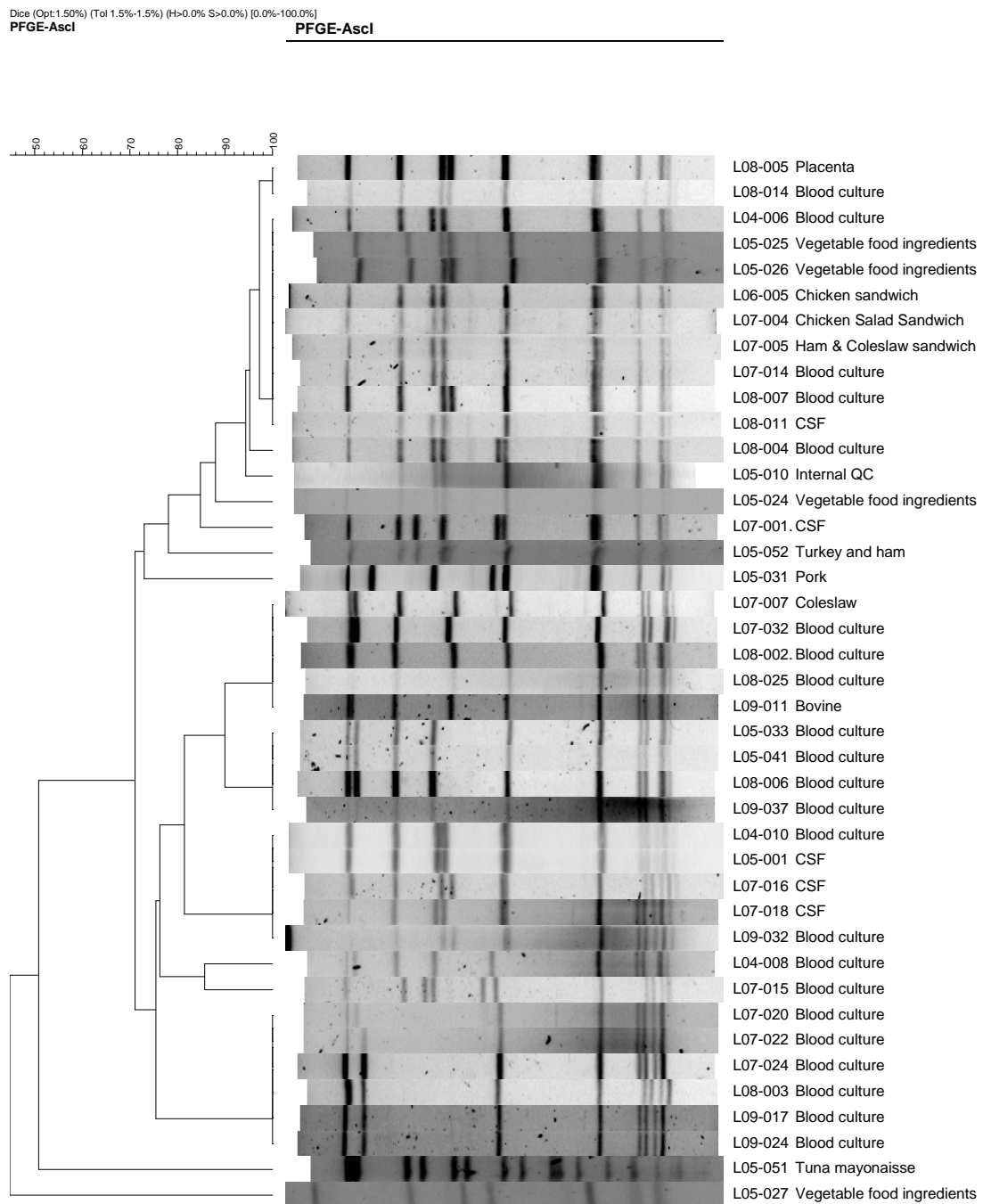
False-positive *Salmonella* results due to laboratory cross-contamination are a serious problem for laboratories and can be difficult to detect. Cross contamination in a laboratory can result in inappropriate diagnosis of patient infection or in unfounded concerns regarding the safety of a food product. Detailed subtyping of isolates by the NSRL helps in

detection and confirmation of laboratory contamination incidents (Role of Subtyping in Detecting *Salmonella* Cross Contamination in the Laboratory; BMC Microbiology: 9; 155). The NSRL recognised 3 such incidents (3 isolates) with *Salmonella* in 2009 with 2 of the incidents involving the laboratory positive control strain, *S.*Nottingham, and 1 incident involving a *S.*Typhimurium control isolate. The 2 *S.*Nottingham incidents involved food laboratories while the *S.*Typhimurium incident involved a human clinical sample. We would like to reiterate our request that all laboratories involved in testing *Salmonella* from any source use *Salmonella* Nottingham as their positive control.

Other Testing:

Listeria monocytogenes

Fig. 2 Dendrogram of *L.monocytogenes* 4b isolates digested with *Apa*I



The NSRL received 39 *Listeria monocytogenes* isolates in 2009. These included 8 human clinical isolates; 7 from blood cultures and 1 from joint fluid. Four of the isolates typed as serotype 4b while four typed as serotype 1/2. Pulsed field gel electrophoresis (PFGE) with *ApaI* and *AscI* showed a serotype 4b cluster of 2 isolates (L09-017 and L09-024).

Comparison of the 2009 patterns with those from other years showed that there were some instances of matches with human isolates from previous years.

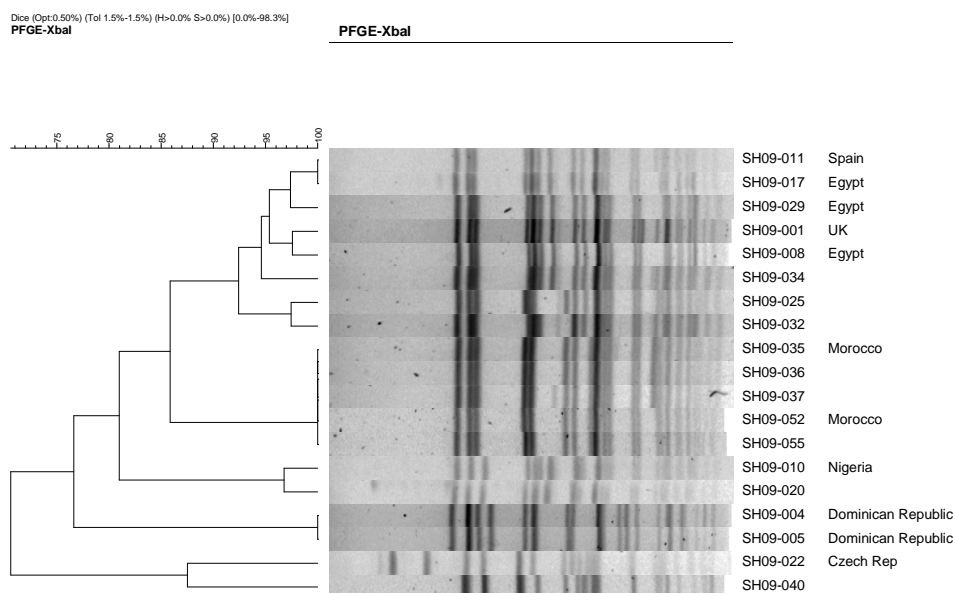
One of the 2009 1/2 isolates (L09-039) was indistinguishable from a human isolate from 2007 (L07-025). Apart from this isolate there was no indication from the molecular typing of any connection between most of the human serotype 1/2 cases and it is likely that they represent isolated sporadic cases.

The majority of the food isolates serotyped as 1/2 (n =29), 1 isolate was serotype 4b and 1 was untypable.

The NSRL is working with colleagues in food and veterinary microbiology in Ireland and with colleagues in Europe to build a library of typing data that may help to identify sources of human infection. A critical limiting factor is the availability of human isolates for typing. The total number of human clinical isolates in Ireland per year is very small therefore it is critical that all such isolates are available for typing and we appeal for all isolates to be forwarded for typing.

Shigella species

Fig. 3 Dendrogram of PFGE of *S.sonnei* digested with XbaI



A total of 59 isolates were referred to the NSRL in 2009 for *Shigella* typing. When non-*Shigella*, QC and duplicate isolates were removed a total of 48 *Shigella* isolates were typed. These included 19 *S.sonnei*, 24 *S.flexneri*, 4 *S.dysenteriae* and 1 *S.boydii*. The *S.flexneri* isolates were further divided into 5 *S.flexneri* 1b, 11 *S.flexneri* 2a, 3 *S.flexneri* 1c, 2 *S.flexneri* 2b, 1 *S.flexneri* 3a, and 2 *S.flexneri* 6.

Thirty of these patients had a recorded history of recent foreign travel, including Africa (n = 18), Australasia (n = 5), Europe (n = 5) and the Americas (n = 2).

Table 7: Foreign travel history for *Shigella* isolates

Continent	Country	Number
Africa (n = 18)		
	Egypt	6
	Nigeria	2
	Morocco	2
	S.Africa	2
	Ghana	1
	Tunisia	1
	Algeria	1
	Mozambique	1
	Uganda	1
	Africa *	1
Europe (n = 5)		
	Spain	1
	Portugal	1
	UK	1
	Czech Republic	1
	Northern Europe	1
Australasia (n = 5)		
	Pakistan	3
	India	2
Americas (n = 2)		
	Dominican Republic	2

* Country not stated

PFGE analysis confirmed a *S.sonnei* outbreak (n = 2) associated with travel to the Dominican Republic and a *S.sonnei* outbreak in the Midlands in June (n = 3).

NSRL Publications and Presentations 2009

Poster Presentations

Use of MLVA in the Detection and Linkage of *S.Typhimurium* Outbreaks to Specific Animal and Food Sources.

ASM Conference, Aix-en-Provence, France. Oct 2009.

Comparative Genomic Analysis of *Salmonella enterica* Serovar Enteritidis Typing Phages SETP3, SETP7 and SETP13.

ASM Conference, Aix-en-Provence, France. Oct 2009.

Papers

Boyle, F., Morris, D., O Connor, J., DeLappe, N., Ward, J., and M.Cormican (2010). First Report of Extended-Spectrum-B-Lactamase-Producing *Salmonella enterica* Serovar Kentucky Isolated from Poultry in Ireland. **AAC. 54:551-553.**

De Lappe, N., O Connor, J., Doran,G., Devane,G., and M. Cormican (2009). Role of subtyping in detecting *Salmonella* cross contamination in the laboratory. **BMC Microbiol. 9: 155.**

De Lappe, N., Doran,G., O Connor,J., O'Hare,C., and M. Cormican (2009). Characterization of bacteriophages used in the *Salmonella enterica* serovar Enteritidis phage-typing scheme. **J. Med. Microbiol. 58: 86-93.**

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Emergence of a multidrug resistant *Salmonella* Concord infections in Europe and the United States in children adopted from Ethiopia 2003-2007. **Paed Infect Dis J** 2009;28:814-8 PMID 19710587

Kingsley, R., Msefula, C., Thomson, N., Kariuki, S., Holt, K., Gordon, M., Harris, D., Clarke, L., Whitehead, S., Sangal, S., Marsh, K., Achtman, M., Molyneux, M., Cormican, M., Parkhill, J., Macleanan, C., Heyderman, R., and G. Dougan.
Epidemic multiple drug resistant *Salmonella* Typhimurium causing invasive disease in Sub-Saharan Africa have a distinct genotype. **Genome Res** 2009.; 19:2279-87 PMID 19901036

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